DETERMINATION OF MOISTURE CONTENT OF EXPRESSED PLANT TISSUE FLUIDS[1]

ROSS AIKEN GORTNER AND WALTER F. HOFFMAN

The fact that the physico-chemical properties of plant tissue fluids reflect in many instances the ecological environment of the plant, and that the ability of a plant to exist under widely different environmental conditions appears to depend largely upon its ability to adjust the physico-chemical properties of its tissue fluids to the new environment has only recently been recognized. HARRIS (3) has pointed out that any thorough ecological study should include physico-chemical studies of the plant saps. GORTNER and HARRIS (1) have indicated some of the precautions which must be taken in order to secure an accurate measure of the osmotic pressure of expressed plant saps by the cryoscopic method, and in subsequent papers HARRIS and his co-workers (4, 5, 8–14) have investigated the physico-chemical properties of plant saps in a variety of habitats. The physico-chemical determinations which have been used have necessarily been limited to those which are adapted to field laboratory facilities, and which do not require excessive amounts of either time, apparatus, or plant materials. The determinations have therefore been confined exclusively to the measurement of osmotic pressure by the cryoscopic method, the electrical conductivity by the conventional wheatstone bridge, and more recently hydrogen ion concentration (unpublished data).

Throughout all this work it has been recognized that a knowledge of the moisture content of the expressed plant saps would be most desirable. For example, a knowledge of the total solids combined with the depression of the freezing point would permit the calculation of the "average molecular weight" of the dissolved solutes. An increase in "average molecular weight" in a different environment might logically be interpreted as indicating a response

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to the changed environment by the elaboration of more colloidal materials. In some of the earlier papers (**2, 6, 7**) it was possible to secure these data by the rather laborious procedure of drying weighed portions of the saps in a water oven at 100° and weighing the residue. This method, of course, is wholly unsuited to field studies where hundreds of samples are involved, and is likewise inaccurate, inasmuch as caramelization of the sugars always takes place when plant saps are dried by means of heat. Marked caramelization can only be prevented by drying at room temperature *in vacuo* over sulphuric acid, or by drying in a vacuum oven at not to exceed 50° C. When such methods are employed constant weight is not reached until several days have elapsed.

Another objection to any drying process for field laboratory work lies in the fact that there may be suspended cell débris in expressed plant sap. With abundant laboratory facilities at hand it is comparatively easy to remove such materials by means of a high speed centrifuge or rapid filtration, but in a field laboratory not equipped with a powerful centrifuge, the removal of such débris may be so incomplete as to seriously affect values of dry matter determinations obtained by a drying process.

It recently occurred to one of the writers that it might be possible to determine the moisture content by making use of the refractive index of the plant sap. This method has been employed by sugar manufacturers for many years, and refractometers may be purchased which have a special "sugar scale" from which the percentage of a sugar in a syrup may be read directly.

Tables of refractive indices were consulted and they confirmed this theory, for the refractive indices of solutions of inorganic salts and proteins *in the concentrations normally present in plant saps* appeared to be sufficiently near the values for solutions of carbohydrates so that no excessive errors should result. Accordingly a high grade Abbé refractometer was secured, provided with a special sugar scale, and carefully standardized by the Bureau of Standards, and determinations were made on a series of plant saps with the results shown in table I. This table does not represent selected determinations, but instead every determination which was completed is included, with the exception of two or three where accidents

TABLE I

Leaves of	Refractive index 20° C.	Δ	Moisture by refractometer (percentage)	Moisture over H_2SO_4 *in vacuo*, room temperature (percentage)	Moisture dried 12 hours more at 100° *in vacuo* (percentage)	Moisture dried 6 hours more at 100° *in vacuo** (percentage)	Average molecular weight of solutes
1. Datura Stramonium	1.3433	0.987	92.75	.....	.....	94.18	147
2. Sambucus canadensis	1.3508	1.212	88.00	.....	.....	90.60	210
3. Bryophyllum calycinum	1.3415	0.508	94.10	.....	.....	94.98	230
4. Panicum sp	1.3420	0.984	93.90	.....	.....	94.64	123
5. Zebrina pendula	1.3372	0.370	97.00	98.33	98.42	.....	155
6. Cyperus alternifolia	1.3411	0.956	94.50	95.19	95.55	.....	113
7. Ricinus communis	1.3472	0.747	90.30	90.81	92.39	.....	267
8. Salix sp	1.3571	1.576	84.00	84.34	87.01	.....	227
9. Triticum vulgare var. Marquis	1.3410	0.897	94.50	94.80	95.48	.....	121
10. Triticum vulgare var. Marquis	1.3404	0.857	94.85	94.79	95.51	.....	118
11. Triticum vulgare var. Marquis	1.3409	1.396	94.60	95.09	95.80	.....	77
12. Triticum vulgare var. Marquis	1.3401	0.889	95.00	94.63	95.60	.....	110
13. Triticum vulgare var. Turkey	1.3533	1.276	86.50	86.85	.....	88.94	230
14. Triticum vulgare var. Buffum	1.3561	1.445	84.60	84.67	.....	87.33	236
15. Bryophyllum calycinum	1.3414	0.474	94.10	94.40	.....	95.01	246
16. Cereus sp	1.3400	0.505	95.10	95.27	.....	.....	190
17. Triticum vulgare var. Buffum	1.3600	1.719	82.20	82.85	.....	85.59	234
18. Triticum vulgare var. Minhardi	1.3455	1.147	91.50	91.80	.....	92.62	150
19. Triticum vulgare var. Super	1.3434	1.000	92.90	93.15	.....	93.88	142
20. Triticum vulgare var. Super	1.3474	1.085	90.30	91.53	.....	.....	184
21. Triticum vulgare var. Super	1.3346	.....	98.85	98.86	.....	.....	.....

* Nos. 1, 2, 3, 4 were dried directly in vacuum oven.

happened to the duplicate set being dried either over sulphuric acid or in the vacuum oven.

The tissue fluids were obtained by means of a specially constructed press bowl, and a hydraulic press, after the tissue had been rendered permeable by a preliminary freezing of the tissue for at least eight hours, following the procedure recommended by GORTNER and HARRIS (1) and used in all of the previous work. All saps were centrifuged perfectly clear from suspended débris. We have included in the table values for Δ, the depression of the freezing point (corrected for under cooling), and the "average molecular weight"[1] of the solutes.

Certain of the samples (nos. 9–14 and 17–21) were collected for another purpose by Mr. ROBERT NEWTON, and the significance of the various values will be discussed by him in a later paper. It is sufficient for our purpose to point out the difference between nos. 11 and 14. We have here two wheat saps differing approximately 3 per cent in the freezing point depression (and consequently in osmotic pressure), and at the same time differing by nearly 300 per cent in total solids. This difference can only be due to a difference in colloidal content, a fact that has been proved by NEWTON, using several other methods. Had we been concerned only with determinations of osmotic pressure, electrical conductivity, and hydrogen ion concentration, we might have concluded that these saps possessed practically identical physico-chemical properties, whereas such a conclusion is far from the truth.

Sample no. 21 is a wheat sap dialyzed completely free of sugar and electrolytes, and represents the non-dialyzable colloidal material. It will be noted that the refractometric method measures this colloidal material quantitatively. NEWTON recently had occasion to prepare, in this laboratory, gum acacia sols containing 1, 2, 3, 5, 7, and 10 per cent of highly purified gum acacia, and refractometric readings for total solids on the resulting solutions gave values corresponding with those of the weighed gum acacia which had been added to make the sols.

[1] Calculated by aid of published tables. Cf. HARRIS, J. A., and GORTNER, R. A., Tables of the relative depression of the freezing point, $1860/\Delta$, to facilitate the calculation of molecular weights. Biochem. Bull. 3:259–263. 1914.

One great advantage of the proposed method is that only two or three drops of sap are required for the determination. A film of sap is placed upon the prism, the prism is closed, and as soon as the thermometer inclosed within the prism has reached 20° C. the reading of the moisture content can be made. The entire procedure need not take more than two minutes.

The column "Moisture by refractometer" is read direct from the scale of the instrument, the next column was obtained by weighing 10–20 gm. of the sap into a glass weighing bottle, and drying to constant weight at room temperature in a vacuum desiccator over sulphuric acid. The dried residue varied in color from a clear green to light brown, and still retained the characteristic odor of plant sap.

The next two columns were obtained by heating the residues from the sulphuric acid vacuum treatment in a Freas vacuum oven at 100° under a vacuum of 28 inches for the stated period of time, and again weighing. It will be noted that in each instance there is a higher moisture content indicated by the further drying in the vacuum oven. We believe this to be almost entirely due to the decomposition of carbohydrates, since there was always marked browning or blackening of the residue, and a pronounced burned sugar odor. In no instance was the residue again wholly water soluble, and in every instance the water extracts of the residue were dark opaque brown, with a strong caramel odor. Further proof that this loss of weight is due to decomposition of carbohydrates is afforded by the fact that the loss in weight continues for a long time, and constant weight is in many instances not reached even after seventy-two hours' drying in the vacuum oven at 100° C.

It will likewise be noted that in most instances the refractometer indicates slightly more total solids (that is, less water) than does the drying over sulphuric acid *in vacuo*. In every instance the sulphuric acid in the vacuum desiccator darkened as the drying progressed, indicating that volatile organic compounds were dissolving in the sulphuric acid. It is self evident that esters, alcohols, ethers, and volatile oils are present in all plant saps, and none of these would be estimated by any drying method. We believe, therefore, that at least a part of the excess total solids indicated by the new method are due to such volatile compounds, and that the

refractometer reading more nearly expresses the true value of the moisture content than can be obtained by any known method.

UNIVERSITY FARM
ST. PAUL, MINN.

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